

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application:

1. (Original) An isolated nucleic acid molecule engineered to comprise all or a portion of at least two Ter sites, wherein the nucleic acid comprises an origin of replication and the Ter sites are arranged with respect to the origin of replication such that the sequence between the two Ter sites is not replicated.
2. (Original) The nucleic acid molecule of claim 1, at least one Ter site is selected from a group consisting of TerA, TerB, TerC, TerD, TerE, TerF, TerG, TerH, TerI, and TerJ.
3. (Original) The nucleic acid molecule of claim 1, wherein the molecule comprises all or a portion of a TerB site.
4. (Original) The nucleic acid molecule according to claim 1, wherein the nucleic acid molecule is selected from a group consisting of plasmids, transposons, BACs, YACs, and phages.
5. (Original) The nucleic acid molecule according to claim 1, wherein the molecule is a linear molecule comprising all or a portion of a Ter site capable of being bound by a Ter-binding protein at each end.
6. (Original) The molecule according to claim 1, further comprising one or more sequences selected from a group consisting of recombination sequences, restriction enzyme recognition sequences, topoisomerase sites, promoters, enhancers, tag sequences and selectable marker sequences.
7. (Original) The nucleic acid molecule according to claim 6, wherein the recombination site is a site specific recombination site.

8. (Original) The nucleic acid molecule according to claim 7, wherein the recombination site is an att site.
9. (Original) The nucleic acid molecule according to claim 8, wherein the att site comprises a sequence of Table 3.
10. (Original) A modified Ter-binding protein.
11. (Currently amended) The protein according to claim 10, wherein the Ter-binding protein comprises all or a portion of a replicator terminator protein (RTP) ~~one or more sequences selected from the group consisting of the sequences in Tables 5-14.~~
12. (Original) The protein according to claim 10, wherein the modification comprises at least one polypeptide.
13. (Original) The protein according to claim 10, wherein the modification is a fusion or insertion of all or a portion of a protein sequence.
14. (Original) The protein according to claim 13, wherein the modification is selected from a group consisting of green fluorescent protein, alkaline phosphatase, horseradish peroxidase, beta-galactosidase, luciferase and beta-glucuronidase.
15. (Original) The protein according to claim 10, wherein the modification comprises one or more molecules selected from a group consisting of comprises a fluorescent molecule, a chromophore, and a radiolabel.
16. (Original) A support comprising at least one oligonucleotide that comprises all or a portion of a Ter site.

17. (Original) The support according to claim 16, wherein the support is a non-biological material.
18. (Original) The support according to claim 16, wherein the oligonucleotide is capable of forming a stem-loop or hairpin.
19. (Original) The support according to claim 16, wherein a duplex portion of a stem-loop or hairpin comprises all or a portion of a Ter site.
20. (Original) A support comprising all or a portion of a Ter-binding protein.
21. (Original) The support according to claim 20, wherein solid support is a non-biological material.
22. (Currently amended) The support according to claim 20, wherein the Ter-binding protein comprises all or a portion of a replicator terminator protein (RTP)~~one or more sequences selected from the group of sequences of Tables 5-14.~~
23. (Original) A method for directional cloning, comprising: providing a nucleic acid molecule comprising one or more Ter sites or portions thereof; providing a vector molecule comprising one or more Ter sites or portions thereof; inserting the nucleic acid molecule into the vector molecule; and selecting the vector molecule comprising the nucleic acid molecule in the desired orientation.
24. (Original) The method according to claim 23, wherein the selecting step comprises transfecting the vector molecule into a host cell, wherein the host cell expresses a Ter-binding protein.
25. (Original) The method according to claim 24, wherein the Ter-binding protein comprises all or a portion of one or more sequences selected from the group of sequences of Tables 5-14.
26. (Original) The method according to claim 23, wherein selecting comprises inhibiting replication of the vector molecule comprising the nucleic acid molecule in an undesired orientation.

27. (Original) The method according to claim 23, wherein the Ter site or sites in the nucleic acid molecule and the Ter site or sites in the vector are partial Ter sites.
28. (Original) A method for attaching a nucleic acid to a solid support, comprising: attaching all or a portion of one or more Ter-binding proteins to a solid support; and contacting the Ter-binding protein with a first nucleic acid, said nucleic acid comprising a Ter site.
29. (Original) The method according to claim 28, wherein the Ter-binding protein comprises all or a portion of one or more sequences selected from the group of sequences of Tables 5-14.
30. (Original) The method of claim 28, further comprising contacting the first nucleic acid with a second nucleic acid.
31. (Original) A method of improving the transfection efficiency of a nucleic acid molecule, comprising: providing all or a portion of one or more Ter site in the nucleic acid molecule; and contacting the nucleic acid molecule with all or a portion of one or more Ter-binding proteins.
32. (Original) The method according to claim 31, wherein the Ter-binding protein is a modified Ter-binding protein.
33. (Original) The method according to claim 31, wherein the Ter-binding protein comprises a receptor binding ligand.
34. (Original) The method according to claim 31, wherein the Ter-binding protein comprises a cellular targeting sequence.
35. (Original) The method according to claim 31, wherein the Ter-binding protein comprises a cell surface binding component.

36. (Original) The method according to claim 34, wherein the cellular targeting sequence is a nuclear localization sequence.
37. (Original) A composition comprising a nucleic acid molecule according to claim 1 and comprising a Ter-binding protein.
38. (Original) A composition according to claim 37, wherein the Ter-binding protein comprises all or a portion of one or more sequences selected from the group of sequences of Tables 5-14.
- 39 to 41 (Cancel)
42. (Original) A method for detecting a biological molecule, comprising: contacting a biological molecule with a reagent, said reagent comprising a nucleic acid portion and a portion that is capable of forming a specific complex with the biological molecule to form a detection mixture; contacting the detection mixture with a nucleic acid binding protein comprising a detection molecule, wherein the nucleic acid binding protein specifically binds to the nucleic acid portion of the reagent; and determining the presence or absence of the detection molecule in the detection mixture, wherein presence of the detection molecule correlates to presence of the biological molecule and absence of the detection molecule correlates to absence of the biological molecule.
43. (Original) The method according to claim 42, wherein the nucleic acid portion of the reagent comprises all or a portion of one or more Ter sites.
44. (Original) The method according to claim 42, wherein the nucleic acid binding protein comprises all or a portion of one or more is Ter-binding proteins.
45. (Original) The method according to claim 42, wherein the detection molecule is selected from the group consisting of radiolabels, epitopes, haptens, mimetopes, affinity tags, aptamers, chromophores, fluorophores and enzymes.

46. (Original) The method according to claim 42, wherein the detection molecule is selected from the group consisting of green fluorescent protein, horseradish peroxidase, alkaline phosphatase, beta galactosidase, beta glucuronidase and luciferase.
47. (Original) A composition comprising all or a portion of one or more Ter-binding proteins attached to a support.
48. (Original) The composition of claim 47, wherein the support is a non-biological material.
49. (Currently amended) The composition according to claim 47, wherein the Ter-binding protein comprises all or a portion of a replicator terminator protein (RTP) ~~one or more sequences selected from the group consisting of the sequences in Tables 5-14.~~
50. (Original) The composition according to claim 47, wherein the support is a bead.
51. (Original) The composition according to claim 47, wherein the support is a chromatography medium.
52. (Original) The composition according to claim 47, wherein the support is a filter or membrane.
53. (Original) A method for separating a nucleic acid containing all or a portion of one or more Ter sites from a mixture, comprising: contacting the nucleic acid with a composition comprising all or a portion of a one or more Ter-binding proteins, wherein the Ter-binding protein binds to the Ter site; and separating the bound nucleic acid from the mixture.
54. (Original) A method according to claim 53, wherein the Ter-binding protein is attached to a support.
55. (Original) The method according to claim 53, wherein the Ter-binding protein comprises all or a portion of one or more sequences selected from the group of sequences of Tables 5-14.

56. (Original) The method according to claim 53, wherein the mixture comprises at least one nucleic acid that is not bound by a Ter-binding protein, and further comprising isolating the nucleic acid that is not bound by the Ter-binding protein.
57. (Original) The method according to claim 53, wherein separating comprises contacting the bound Ter-binding protein with an antibody that specifically binds to the Ter-binding protein.
58. (Original) The method according to claim 57, wherein the antibody is bound to a solid support.
59. (Original) The method according to claim 53, further comprising isolating the bound nucleic acid.
60. (Original) A kit comprising one or more molecules selected from the group consisting of a nucleic acid molecule engineered to comprise all or a portion of at least two Ter sites and a polypeptide comprising all or a portion of one or more Ter-binding proteins.
61. (Original) The kit according to claim 60, further comprising one or more nucleotides, one or more DNA polymerases, one or more reverse transcriptases, one or more suitable buffers, one or more primers, instructions, or one or more terminating agents.
62. (Original) The kit according to claim 60, wherein said nucleic acid molecule further comprises at least one recombination site.
63. (Original) The kit according to claim 62, wherein said recombination site is selected from the group consisting of att sites and lox sites.
64. (Original) The kit according to 62, further comprising at least one recombination protein.
65. (Original) The kit according to claim 64, wherein the recombination protein is selected from the group consisting of integrase, Cre, IHF, Xis, Flp, Fis, Hin, Gin, .PHI.C31, Cin, Tn3 resolvase, TndX, XerC, XerD, TnpX, Hjc, Gin, SpCCE1, and ParA.

66. (Original) The kit according to claim 65, wherein the recombination protein is integrase.
67. (Original) A method of juxtaposing a Ter site on a nucleic acid molecule with a second site on the nucleic acid molecule, comprising: providing a nucleic acid molecule having a Ter site; contacting the nucleic acid with a Ter-binding protein in functional association with an enzyme capable of translocating along the nucleic acid molecule; and conducting a reaction that causes the enzyme to translocate, thereby juxtaposing the Ter site and the second site.
68. (Original) The method of claim 67, wherein the nucleic acid comprises a promoter in proximity to the Ter site and the enzyme is a polymerase.
69. (Original) A method of cloning, comprising: providing a linear vector comprising a portion of a Ter site on each end; ligating a nucleic acid of interest with the vector to form a ligation mixture, wherein vectors that do not ligate with a nucleic acid reform a functional Ter site; and introducing the ligation mixture into host cells, wherein host cells that receive a vector with a functional Ter site do not replicate the vector.
70. (Original) A method for synthesizing a double stranded nucleic acid molecule comprising all or a portion of one or more Ter sites, comprising: (a) mixing one or more nucleic acid templates with a polypeptide having polymerase activity and one or more primers comprising all or a portion of one or more Ter sites; (b) incubating said mixture under conditions sufficient to synthesize a first nucleic acid molecule which is complementary to all or a portion of said templates and which comprises said all or portion of one or more Ter sites; and (c) incubating said first nucleic acid molecule in the presence of one or more primers under conditions sufficient to synthesize a second nucleic acid molecule complementary to all or a portion to said first nucleic acid molecule, thereby producing a double stranded nucleic acid molecule comprising all or a portion of one or more Ter sites.
71. (Original) The method of claim 70, wherein all or a portion of at least one Ter site is located at or near one terminus of said double stranded nucleic acid molecule.

72. (Original) The method of claim 70, wherein said template is RNA or DNA.
73. (Original) The method of claim 70, wherein said template comprises one or more polyA RNA molecules.
74. (Original) The method of claim 73, wherein said polyA RNA molecules are mRNA molecules.
75. (Original) The method of claim 70, wherein said polypeptide is selected from the group consisting of a reverse transcriptase, a DNA polymerase, and combinations thereof.
76. (Original) The method of claim 75, wherein said DNA polymerase is a thermostable DNA polymerase.
77. (Original) The method of claim 76, wherein said thermostable DNA polymerase is selected from the group consisting of *Thermus thermophilus* (Tth) DNA polymerase, *Thermus aquaticus* (Taq) DNA polymerase, *Thermotoga neopolitana* (Tne) DNA polymerase, *Thermotoga maritima* (Tma) DNA polymerase, *Thermococcus litoralis* (Tli or VENT.RTM.) DNA polymerase, *Pyrococcus furiosus* (Pfu or DEEPVENT.RTM.) DNA polymerase, *Pyrococcus woosii* (Pwo) DNA polymerase, *Bacillus sterothermophilus* (Bst) DNA polymerase, *Sulfolobus acidocaldarius* (Sac) DNA polymerase, *Thermoplasma acidophilum* (Tac) DNA polymerase, *Thermus flavus* (Tfl/Tub) DNA polymerase, *Thermus ruber* (Tru) DNA polymerase, *Thermus brockianus* (DYNAZYME.RTM.) DNA polymerase, and *Methanobacterium thermoautotrophicum* (Mth) DNA polymerase.
78. (Original) The method of claim 70, further comprising amplifying said first and second nucleic acid molecules.
79. (Original) The method of claim 78, wherein said amplification is accomplished by a method comprising (a) contacting said first nucleic acid molecule with a first primer which is complementary to a portion of said first nucleic acid molecule, and a second nucleic acid molecule

with a second primer which is complementary to a portion of said second nucleic acid molecule with a polypeptide having polymerase activity; (b) incubating said mixture under conditions sufficient to form a third nucleic acid molecule complementary to all or a portion of said first nucleic acid molecule and a fourth nucleic acid molecule complementary to all or a portion of said second nucleic acid molecule; (c) denaturing said first and third and said second and fourth nucleic acid molecules; and (d) repeating steps (a) through (c) one or more times, wherein said first primer and/or said second primer comprise all or a portion of one or more Ter sites.

80. (Original) A method for synthesizing a double stranded nucleic acid molecule comprising: mixing one or more nucleic acid templates with a polypeptide having polymerase activity and one or more primers comprising all or a portion of at least a first Ter site; incubating said mixture under conditions sufficient to synthesize a first nucleic acid molecule which is complementary to all or a portion of said one or more templates and which comprises at least said all or portion of a first Ter site; and incubating said first nucleic acid molecule in the presence of one or more primers under conditions sufficient to synthesize a second nucleic acid molecule complementary to all or a portion to said first nucleic acid molecule, thereby producing a double stranded nucleic acid molecule comprising all or a portion of at least a first Ter site, wherein said all or portion of a first Ter site comprises at least one nucleotide sequence that has at least 80-99% homology to a nucleotide sequence selected from the group of sequences in Table 4 and a corresponding or complementary DNA or RNA sequence.
81. (Original) The method of claim 80, wherein said all or portion of a Ter site is located at or near one terminus of said double stranded nucleic acid molecule.
82. (Original) The method of claim 80, further comprising amplifying said first and second nucleic acid molecules.
83. (Original) A method for adding one or more Ter sites or portions thereof to one or more nucleic acid molecules, said method comprising: (a) contacting one or more nucleic acid molecules with one or more integration sequences which comprise one or more Ter sites or portions thereof; and (b)

incubating said mixture under conditions sufficient to incorporate said integration sequences into said nucleic acid molecules.

84. (Original) The method of claim 83, wherein said integration sequences are selected from the group consisting of transposons, integrating viruses, integrating elements, integrons and recombination sequences.
85. (Original) The method of claim 83, wherein at least one nucleic acid molecule is genomic DNA.
86. (Original) A method for producing one or more cDNA molecules or a population of cDNA molecules comprising (a) mixing an RNA template or population of RNA templates with a reverse transcriptase and one or more primers wherein said primers comprise one or more Ter sites or portions thereof; and (b) incubating said mixture under conditions sufficient to make a first DNA molecule complementary to all or a portion of said template, thereby forming a first DNA molecule comprising one or more Ter sites or portions thereof.
87. (Original) A method for synthesizing one or more nucleic acid molecules comprising all or a portion of one or more Ter sites, said method comprising: (a) obtaining one or more linear nucleic acid molecules; and (b) contacting said molecules with one or more adapters which comprise one or more Ter sites or portions thereof under conditions sufficient to add one or more of said adapters to one or more termini of said linear nucleic acid molecule.
88. (Original) A nucleic acid molecule comprising all or a portion of a Ter site flanked by recombination sites.
89. (Original) A nucleic acid molecule according to claim 88, wherein the recombination sites are selected from a group consisting of att sites, lox sites, and FRT sites.
90. (Original) A nucleic acid molecule according to claim 88, wherein the Ter site is selected from a group consisting of the Ter site sequences in Table 4.

91. (Original) A method of cloning two DNA fragments into one vector in one reaction, wherein said vector comprises two markers for negative selection, said method comprising: replacing a first marker for negative selection with a first DNA fragment; in the same reaction mixture, replacing a second marker for negative selection with a second DNA fragment; and transforming host cells that are not resistant to either negative selection.
92. (Original) The method of claim 91, wherein recombination is used to replace at least one of said markers for negative selection.
93. (Original) The method of claim 92, wherein said recombination is site-specific recombination.
94. (Original) The method of claim 93, wherein said site-specific recombination is mediated by a recombination protein selected from the group consisting of integrase, Cre, IHF, Xis, Flp, Fis, Hin, Gin, .PHI.C31, Cin, Tn3 resolvase, TndX, XerC, XerD, TnpX, Hjc, Gin, SpCCE1, and ParA.
95. (Original) The method of claim 91, wherein said first DNA fragment and said second DNA fragment encode proteins that interact with each other.
96. (Original) The method of claim 91, wherein said first DNA fragment and said second DNA fragment encode proteins that are part of the same metabolic pathway.
97. (Original) The method of claim 91, wherein said first DNA fragment and said second DNA fragment encode proteins that are part of the same signaling pathway.
98. (Original) The nucleic acid of claim 1, wherein said nucleic acid is selected from the group consisting of pTER1, pTER2 and pTER3.

99. (New) The support according to claim 21, wherein the support comprises one or more of silicon, biochips, nitrocellulose, diazocellulose, glass, polystyrene, polyvinylchloride, polypropylene, polyvinylidenedifluoride (PVDF), dextran, Sepharose, agar, starch, nylon, polymerized Langmuir Blodgett film, functionalized glass, Si, Ge, GaAs, GaP, SiO₂, SiN₄, modified silicon, (poly)tetrafluoroethylene, (poly)vinylidenedifluoride, polystyrene, or polycarbonate.
100. (New) The support according to claim 48, wherein the support comprises one or more of silicon, biochips, nitrocellulose, diazocellulose, glass, polystyrene, polyvinylchloride, polypropylene, polyvinylidenedifluoride (PVDF), dextran, Sepharose, agar, starch, nylon, polymerized Langmuir Blodgett film, functionalized glass, Si, Ge, GaAs, GaP, SiO₂, SiN₄, modified silicon, (poly)tetrafluoroethylene, (poly)vinylidenedifluoride, polystyrene, or polycarbonate.